Amendment

In the Specification:

Please amend the specification in accordance with 37 C.F.R. § 1.77 to include the following abstract, which is attached herewith on a separate sheet:

The invention provides novel Ti-plasmid vectors for *Agrobacterium*-mediated plant cell transformation. Plamsids of the invention are constructed with a DNA sequence (*i.e.*, a negative selectable marker or a DNA sequence that inhibits DNA unwinding) flanking the T-DNA bordered gene of interest that reduces or eliminates the occurrence of transformants with vector read-through DNA sequence. The invention also provides methods of plant cell transformation utilizing these novel vectors.

On page 1, line 1, please delete the title and add the following:

C1 SELECTIVE TI-PLASMIDS AND METHODS OF USING THE SAME

On page 1, line 12, please delete the title and add the following:

Description of the Related Art

On page 2, line 26, please delete the title and add the following:

Brief Summary of the Invention

On page 9, lines please delete the third full paragraph and replace with the following paragraph:

C2 Transformation of plant species is now routine for an impressive number of plant species, including both the *Dicotyledoneae* as well as the *Monocotyledoneae*. In principle any transformation method may be used to introduce chimeric DNA

according to the invention into a suitable ancestor cell. A preferred method according to the invention comprises *Agrobacterium*-mediated DNA transfer. Especially preferred is the use of the so-called binary vector technology (as disclosed in EP A 120 516 and U.S. Patent 4,940,838).

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Tomato transformation is preferably done essentially as described by Van Roekel et al. (Van Roekel, J.S.C., Damm, B., Melchers, L.S., Hoekema, A. (1993)). Factors influencing transformation frequency of tomato (*Lycopersicon esculentum*). (Plant Cell Reports, 12, 644-647). Potato transformation is preferably done essentially as described by Hoekema et al. (Hoekema, A., Huisman, M.J., Molendijk, L., van den Elzen, P.J.M., and Cornelissen, B.J.C. (1989)). The genetic engineering of two commercial potato cultivars for resistance to potato virus (X. Bio/Technology 7, 273-278).

On page 11, please delete the first and second full paragraphs and replace with the following:

A 40 bp GC-rich stretch was created by annealing SEQ ID NO:5 and -6 to each other. Insertion of this fragment into a SalI site will leave a SalI site at only one end intact. The double stranded synthetic oligo was phosphorylated by T4 polynucleotide kinase and cloned into the SalI-digested pNE03 vector. The resulting plasmid pNE07 has the GC-rich stretch inserted at the SalI site, which results in removal of the SalI site at the side nearest the left T-DNA border. A schematic representation of the orientation is presented in Fig. 1

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The fragment containing VirG binding sites is derived from the VirB promoter of Agrobacterium strain EHA 101. The VirB promoter was previously shown to contain two vir-box sequences which are both recognized by VirG (Das and Pazour, 1989, Nucl. Acids Res. 17, 4541-4150). The Vir-box alone is thought not to be sufficient for binding of the VirG protein, additional specific nonconserved sequences 3' to the Vir-box, approximately 19 bp, are most likely also required for binding of the VirG protein. The primers SEQ ID NO: 7 and -8 were used for PCR amplification of an appr. 90 bp VirB promoter fragment from Agrobacterium tumefaciens strain MOG101. The fragment was digested with SalI and AvaI, and introduced into the unique Sal I site of the pNE03 vector. Again this fragment is oriented so that the SalI-AvaI ends are joined closest to the Left Border. A schematic representation of the orientation is presented in Fig. 1. This vector is denominated pNE09.

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On page 12, please delete the first full paragraph and replace with the following paragraph:

An EcoRI-HindIII fragment from pMOG1059 contained a GUS expression cassette containing 1) the FdrolD chimeric promoter and untranslated sequences (Patent Appl. No. 97203912.7 filed 12/12/97), 2) a GUS Open Reading Frame containing an StLS1 intron (Vancanneyt et al., 1990, Mol. Gen. Genet. 220-245-250) and 3) 3' untranslated/ terminator sequences of the proteinase inhibitor II gene (Thornburg et al., 1987, Proc. Natl. Acad. Sci. USA 84, 744-748). This EcoRI-HindIII fragment was inserted into the EcoRI-

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HindIII sites of binary vectors pNE10, -11 and -12 between the borders. The GUS cassette is closest to the Right Border, the nptII selection marker cassette is found closest to the Left Border (see Fig 1). As an unmodified control pMOG1059 was used, of which the vector sequences are the unmodified pMOG800 backbone. pMOG1313 is the binary vector that has the FdrolD-GUS cassette on the T-DNA and contains the GC clamp next to its left border, pMOG1314 identical within the T-DNA, but contains the virG binding sites next to the left border, pMOG1315 again has the same T-DNA sequences and contains the barnase cassette next to the left border. Likewise, pMOG1316 contains both the GC clamp and the barnase cassette and pMOG1317 the virG binding sites followed by the barnase cassette.

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On page 13, please delete the first full paragraph and replace with the following:

Potato stem segments of cv. Kardal were transformed with *Agrobacterium tumefaciens* strain EHA 105 in three separate transformation experiments using a standard transformation protocol (as described in PCT/EP 98/02979). Per construct a minimum of 150 explants were used. Usually this will lead to regeneration of about 90 transformants/construct. Transformation frequency was determined as the number of regenerants able to root under selective pressure on kanamycin-containing growth medium relative to the number of explants used. For Table 2 we

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normalized the transformation frequency to 1.0 for the control construct pMOG1059.

On page 13, please delete the second full paragraph and replace with the following:

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Table 2. Average relative transformation frequencies observed with constructs tested. All values were normalized per transformation experiment to pMOG1059 (set at 1.00). The values were averaged from three independent transformation experiments.

On page 14, please delete the first full paragraph and replace with the following:

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Next we analyzed the presence of DNA fragments spanning the left and right T-DNA borders by PCR, fragments indicative of integration of vector DNA in transformants. Per construct, 75 individual lines were analyzed for outer border sequences by a multiplex PCR. Six primers were used for the multiplex PCR with npt II primers as an internal control. For the location of primers on the binary vectors see Fig 2.

On page 14, please delete the third full paragraph and replace with the following:

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After amplification the obtained PCR fragments were electrophoresed on a 0.8% agarose gel containing Ethidium Bromide. After photography the different fragments were counted and the percentage of readthrough determined (see Table 3 for compilation).